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Rheological Properties of the Schizophyllan−Borax System

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ABSTRACT: Schizophyllan, a neutral exopolysaccharide, was found to form physical gels in the presence of borate ions. The sol-to-gel transition was studied through rheological experiments. Both the concentration and the nature of added salt were examined and kinetics data are given.

Introduction

Gels, known as an intermediate state between liquid and solid states, are very interesting materials, as they are used in a wide range of applications in everyday life (cosmetics, food industry, pharmaceutics, ...). For many years, efforts have been made for having a comprehensive picture of the structure and properties of gels: a deeper knowledge of such systems will allow us to establish structure−property relations as well as to permit the production of gels suitable for specific applications.

The sol-to-gel transition of gelifying systems is of primary importance in characterizing the behavior of such materials; it often happens as a phenomenon which appears suddenly and requires the use of well-adapted techniques. For example, when considering the temperature-induced gelification of polymer solutions, the transition takes place at a precise temperature, at which significant deviations are observed in the macroscopic properties.[1]

This "critical" behavior is common in numerous systems undergoing gelification; at the gel time, t_{gel}, the viscoelastic properties abruptly change from an initially liquidlike state to a solidlike one.

In the present paper we report the main features of the sol-to-gel transition of schizophyllan aqueous solutions in the presence of borate ions. In particular, attention is being focused on the rheological properties associated with the transition.

Schizophyllan is a neutral exopolysaccharide from *Schizophyllum commune*.[2] Its chemical structure is identical to scleroglucan: the repeat unit, as indicated in Figure 1, consists of linearly linked β-(1→3)-D-glucose residues with one every three laterally substituted with a β-(1→6)-D-glucose residue. Both polysaccharides show remarkable viscosifying properties in aqueous media due to a secondary semirigid helical structure involving three distinct chains.[3,4] In addition, schizophyllan has potential biomedical applications, for example, as an antitumorous agent[5,6] or for pH-controlled drug delivery systems.[7]

Experimental Section

Material. The biopolymer sample was a shear-degraded native sample kindly supplied by Taito Co. Ltd. Its average molecular weight, $\bar{M}_w$, as determined by light scattering,[8] was found to be about 2×10^6.

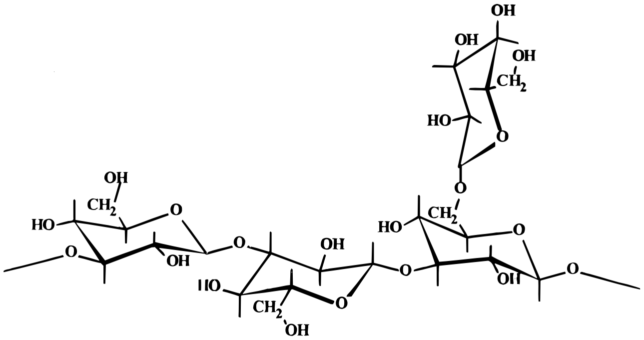

Figure 1. Chemical structure of schizophyllan.

Sodium borate decahydrate $Na_2B_4O_7{\cdot}10H_2O$ (Borax) and the salts used (LiCl, NaCl, KCl, RbCl, and CsCl), all of analytical grade, were from Prolabo.

Preparation of Gels. A semidilute polymer solution and cross-linker solution both at the same salt content were prepared separately at fixed concentrations, corresponding to twice the desired amount of each species in the mixture; thereafter, the pH was adjusted at the desired value using sodium hydroxide. Previous work has clearly demonstrated the primary importance of pH as it governs the cross-linker (namely borate ions) content in the media; it has been established that no gelation occurs for a value lower than pH 8, as the borate ion concentration is then insufficient. Therefore, in the present work, the media was buffered at a pH of 9.2 using Borax. Solutions were allowed to stand for 30 min in a temperature controller bath to reach thermal equilibrium, after which equal volumes of each solution were mixed together under vigorous stirring to prevent eventual precipitation due to local overconcentration. This procedure allowed us to follow the kinetics of gelation using rheological techniques. For high cross-linker content, a phase separation was visually observed and attributed to syneresis.

Measuring Methods. Flow and dynamic rheological measurements were performed using a Carri-Med CSL-100 controlled stress rheometer with a cone and plate (radius 4 cm, cone angle 2°) or a double gap concentric cylinder geometry depending on the texture of the material studied. The rheometer was equipped with a solvent trap for preventing any dehydration phenomenon during the experiment, and the temperature was controlled by the Peltier effect (cone and plate), allowing an accuracy of about 0.1 °C, or by an external temperature controller bath for the other geometry. The kinetics of gel formation was followed by measuring the frequency response of viscoelastic parameters (G', G'', tan δ, η^*, ...) over the entire reaction time. To avoid any sample disruption during the reaction of gelation, the linear viscoelastic spectra were measured at a constant strain of less than 30%.

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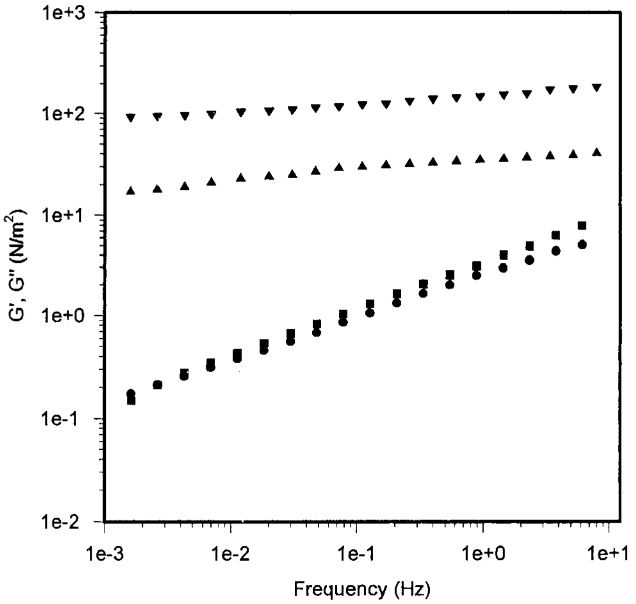

Figure 2. Dynamic moduli for a 6 g/L schizophyllan solution in the absence and in the presence of borax. Solution (C_p = 6/L): (■) G'; (●) G''. Gel (C_p = 6 g/L + 14 mM Borax): (▼) G'; (▲) G''.

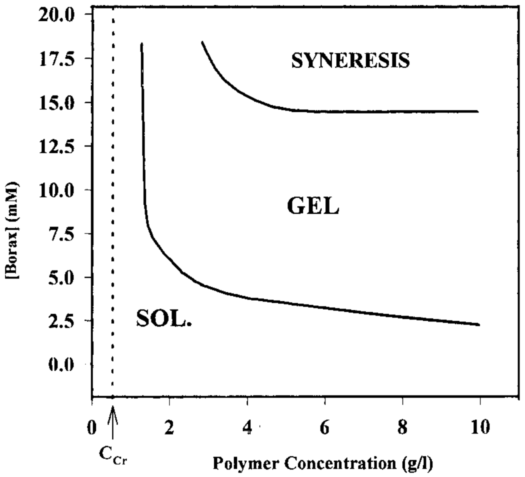

Figure 3. Phase diagram for the schizophyllan–borax system.

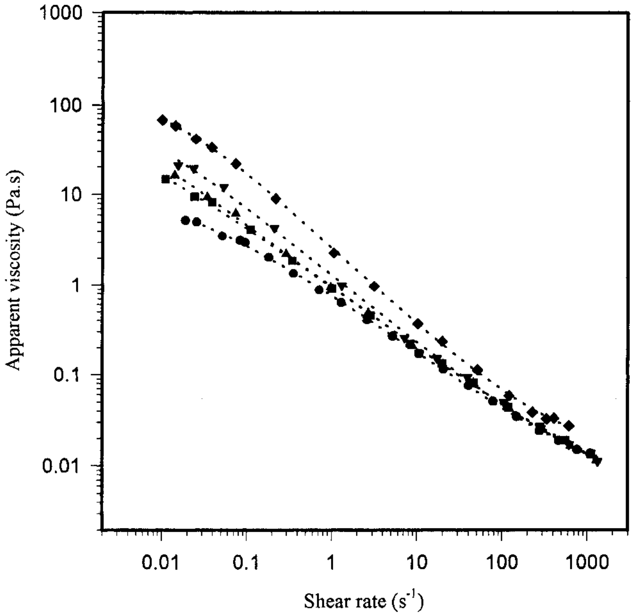

Figure 4. Flow behavior of schizophyllan-borax mixtures at different salt contents. Sodium chloride concentration: (●) 0.25 M; (■) 0.375 M; (▲) 0.5 M; (▼) 0.75 M; and (◆) 1 M.

Results and Discussion

In a previous paper we have reported that, in suitable conditions of concentrations and salinity, schizophyllan was able to give physical gels in the presence of borate ions.[9] Figure 2 illustrates how the frequency dependence of storage (G') and loss (G'') moduli is changed on adding Borax to schizophyllan. In the presence of Borax, the plots of G' and G'' are quite linear and their profiles are those typical of a physical gel according to Almdal et al.[10] In the frequency range of experiment, G' is larger than G'' and shows a pronounced plateau extending to long times. The viscoelastic behavior of the schizophyllan–borax mixtures is strongly affected by the presence of added salt. Initially neutral, the polysaccharide becomes charged as a result of the complexation of borate ions by the diol sites of the polymer chains. As a result, electrostatic repulsions between polymer chains should exist so that, in pure water, the formation of intermolecular dicomplexes is impeded and therefore allowing the establishment of a tridimensional network. Screening of electrostatic repulsion by adding salt is a prerequisite for network formation. A typical phase diagram is given in Figure 3 for a system in the presence of 0.5 mol/L of sodium chloride at 25 °C. The limits between the different phases (gel domain, two-phase region, and sol state) appears much less evident in reality than shown in the figure, and the lines represent averages from many measurements and observations. The gel domain is narrow and for the highest Borax content, syneresis takes place because of collapse of the network due to over-cross-linking. When the schizophyllan concentration is lower than the critical overlapping concentration, no gel formation is observed, as in such conditions there are not enough intermolecular connecting points, as we already mentioned in a previous article.[11] It has been observed that pH acts not only by governing the extent of borate ions in the media but also by affecting the phase separation. The syneresis limit is shifted to lower Borax content when the pH is increased, this being consistent with the effective cross-linker concentration in the media.

Finally, the phase separation occurrence made it necessary to check that the mixture corresponded to a unique phase before performing any rheological measurements.

The behavior of the schizophyllan–borax mixtures at a polymer concentration near and lower than the critical overlapping concentration will be detailed in a following paper.

Figure 4 illustrates the dependence of the flow properties of mixtures on the concentration of added salt. In conditions where the polymer and the Borax contents remain constant, increasing salt concentration results in an increase in low shear viscosity, whereas at high shear rate the viscosity is nearly independent of the salinity. The shear-dependent behavior is markedly shear-thinning and can be explained in terms of destruction of intermolecular low-energy bonds established through borate ions. This behavior is typical of structured fluids or weak gels.

The results presented above clearly indicate that the salt content governs the stability of the intermolecular

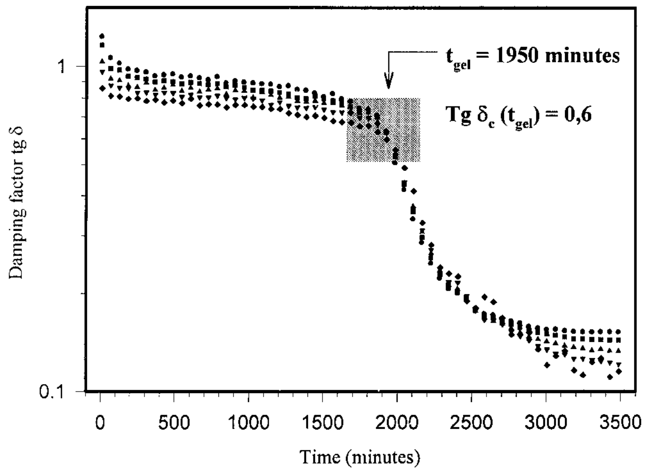

Figure 5. Determination of the critical exponents predicted by the percolation theory using multifrequency experiments. Frequency (Hz): (●) 0.232; (■) 0.387; (▲) 0.646; (▼) 1.078; (◆) 1.797.

links and therefore is responsible for the weak gel properties of the mixtures.

After the different species are mixed, a delay period exists before gelification begins. Consequently, the formation of the gels can be monitored through dynamical measurements, as has been reported for other gelifying systems as ι-carrageenans,[12] κ-carrageenans,[13] or pectins and alginates in the presence of calcium ions.[14] The estimation of the gel time, t_{gel}, can be monitored by following the evolution of the viscoelastic properties of the system. However, the determination of t_{gel} is not so easy if only one frequency is used during the test, as the sol-to-gel transition does not happen in a well-defined time. As a transition zone exists, a precise determination is possible when using multifrequency experiments; the gel time is the time corresponding to a frequency-independent value for tan δ (G''/G'). Figure 5 shows a typical evolution of the time dependence of tan δ for schizophyllan in the presence of Borax.

The plot of log tan δ against time shows a crossing point for frequencies considered (0.232−1.797 Hz) at around 1950 min after the start of the mixing. Before the gel point, the mixture is a viscoelastic fluid (the rheological properties are then typical of polymer solution) and all the experiments have to be carried out at a fixed strain in order to avoid the sample disruption during the measurement.

At the gel point, the macromolecules are largely interconnected; they form a tridimensional network corresponding to a unique giant macromolecule (the only limit corresponds to the beaker). The sharp sol-to-gel transition is attributed to a connectivity transition of the system, as explained by Winter and Chambon using scaling and self-similarity arguments.[15]

Figure 6 shows the evolution of the frequency dependence of the viscoelastic parameters (G' and G'') at different times during the gelation reaction. The properties of the system do not vary rapidly before the gel time; the elastic and loss moduli are largely dependent on the frequency. The pregel state corresponds to an increase of the intermolecular links: the topology is progressively affected, and the system changes from a viscous solution to a viscoelastic fluid.

The evolution of the rheological properties is interpreted as follows. At the start of the complexation reaction between the borate ions and the diol sites of the polysaccharide chains, the elastic modulus is mainly affected in the terminal zone (low frequencies) as very few intermolecular links take place; the relaxation time of the macromolecules is then affected and shifted to longer times. At the sol-to-gel transition the relaxation time spectrum greatly changes, involving a deep change of the system behavior in a large frequency range.[16,17]

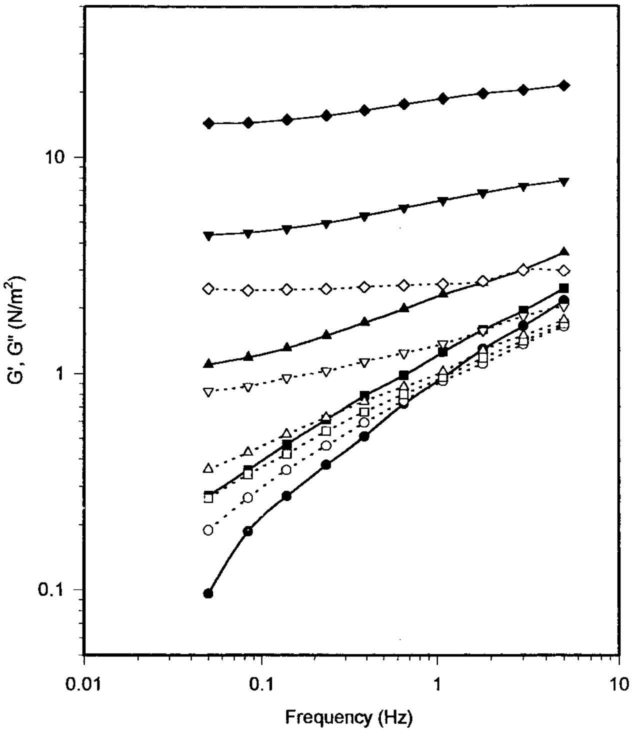

Figure 6. Mechanical spectra of a schizophyllan−borax mixture during the gelification reaction: (●) G', (○) G'' initial; (■) G', (□) G'' 20 h; (▲) G', (△) G'' 35 h (#t_{gel}); (▼) G', (▽) G'' 40 h; (◆) G', (◇) G'' 50 h.

The theory predicts a power law of the elastic and the viscous moduli as a function of the angular frequency with an exponent n (eq 1). This critical exponent can

$$G'(\omega) \sim G''(\omega) \sim \omega^n \tag{1}$$

$$\tan \delta_c = G''(\omega)/G'(\omega) = \text{constant} = \tan(n\pi/2) \tag{2}$$

be determined from tan δ (eq 2). It is interesting to note that mainly for practical reasons (a lot of systems do not permit such determination as the reaction time is not slow enough to allow multifrequency experiments monitoring) but also because of historical considerations,[18] numerous authors often consider the gel point as the time when tan $\delta = 1$ ($G' = G''$). If some disagreement exists concerning the value of the different parameters at the sol-to-gel transition (tan δ_c, exponent n), the gelification reaction is always viewed as an increase in the network connectivity.

Experimentally, the schizophyllan−borax system allows a multifrequency determination of t_{gel}. At the gel point, tan $\delta_c = 0.6$ and the exponent $n = 0.36$ (Figure 5). In Table 1 are listed different values of these parameters for physical as well as chemical gels. From all the experiments we have carried out with the schizophyllan−borax system (at various concentrations of the different species) we found a tan δ_c parameter lying between 0.45 and 0.8. The order of magnitude of this parameter is largely dependent on the different

Table 1. Values of tan δ_c and n for Different Interesting Polymer Systems

system	gel type	tan δ_c	exponent n	ref
ι-carrageenans	physical	0.72	0.42	19
gelatin		1.71	0.69	20
schizophyllan−borax		0.45−0.8	0.34−0.41	this work
PEO−PPO−PEO		n.c.	#0.5	21
PVC−DOP		n.c.	0.77	22
TMOS−methanol	chemical	0.79	0.72	23
polyurethane		1.89	0.69	24
polybutadiene		#1	0.41−0.53	25
PEO		1.7	#0.5	26
alkoxide zirconium		2.21	0.73	27
PGA−TBA		n.c.	0.7	28

Table 2. Effect of Cross-linker Concentration and Salt (Content and Nature) on the Gelation Time of Schizophyllan−Borax Mixtures

cross-linker concn $C_p = 6$ g/L − [NaCl] = 0.5 M − 25 °C		salt concn $C_p = 3$ g/L − [borax] = 10 mM − 25 °C		nature of salt $C_p = 6$ g/L − [borax] = 5 mM − [salt] = 0.5 M	
[borax] (mM)	t_{gel} (min)	[NaCl] (M)	t_{gel} (min)	salt	t_{gel} (min)
2.5	3600	0.25	2200	LiCl	3300
5	3050	0.37	2250	NaCl	3050
8	2300	0.5	1950	KCl	2250
10	1950	0.75	1410	RbCl	2200
14	1100	1	1120	CsCl	2150

species concentration, and whether the system behaves as a physical gel or a viscoelastic fluid. This variation is attributed to variable experimental conditions, and the lowest values are observed when the elasticity of the mixture is nonnegligible because of the polymer concentration (highest schizophyllan concentrations).

Otherwise, the values obtained for the schizophyllan−borax systems are similar to what is usually reported in the literature. This confirms that this approach can be applied to a large panel of systems undergoing sol-to-gel transitions.

Factors Affecting the Sol-to-Gel Transition. Different parameters, namely, concentrations of polymer and cross-linker, concentration and nature of the added salt, and temperature, were investigated. Table 2 shows the effect of changing the experimental conditions over the gel time, t_{gel}; the values given here were determined using multifrequency experiments, as described above. Due to the time required for each experiment, the values reported here are averages of three consecutive tests, provided the difference was less than 5%.

Naturally, the more cross-linker in the medium, the earlier the sol-to-gel transition occurs; the free borate species in the solution will establish more intermolecular links between polysaccharide chains as its concentration is dropped, in accordance with the complexation thermodynamics of such ions. In addition, similar experiments carried out using various schizophyllan concentrations clearly indicated the same trend: as more diol sites susceptible to form intermolecular complexes through the borate ions are present in the medium, the gel formation is favored.

As already mentioned, the polyelectrolyte behavior of the *mono*complexed polymer chains involves strong electrostatic repulsions in the polysaccharide−borax solution, so that the gel state is only observed after screening such effects with added salt.

Results reported in column 2 of Table 2 clearly show that the kinetics of gelation is largely influenced by the added salt concentration (the higher the salt content, the earlier the sol-to-gel transition), indicating that lowering the electrostatic repulsion effects makes easier the complex formation (*mono*- and *di*complexation), which is responsible for the establishment of the gel network.

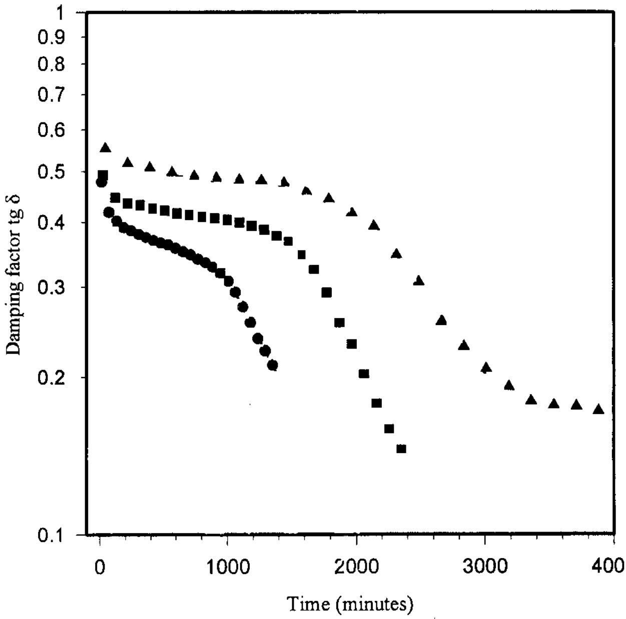

Figure 7. Temperature dependence of the gel point: evolution of the damping factor tan δ during gelification at different temperatures. Temperature (°C): (●) 16.5; (■) 20; (▲) 25.

Finally, as visible in column 3 of Table 2, the size of the counterion has a marked effect on the sol-to-gel transition and the gelation rate is enhanced when the cation size increases ($Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$). The establishment of polymer−borate ion *di*complexes is favored by large counterions, while the transition occurs for longer periods when smaller cations are used: the effect of using larger cations is equivalent to increasing the salt content of the medium.

This effect is explained as the consequence of a better solvation of the polymer−ion complexes when small cations are used: the bound counterion fraction is more important when small cations are considered,[29] so that the aggregation of polymer chains through dicomplexed borate ions is not favored as the result of the *mono*complex stabilization.

Furthermore, considering the osmotic coefficients of the cations from the alkaline series in the presence of polyelectrolytes, Dolar has demonstrated that the degree of binding of counterions becomes higher when the size is lowered,[30] the charges introduced along the polysaccharide chains are therefore better stabilized when the cation becomes smaller, delaying the aggregation reactions between the polymer chains and thus the gel formation.

If the phase diagram is considered, the larger the cation, the lower the cross-linker concentration at the phase separation, this being consistent with the above interpretations.

The observations clearly indicate that solvent quality appears of primary importance for the system behavior, as it governs the privileged interactions taking place in the mixture.

Temperature Effect. Figure 7 illustrates the time dependence of tan δ at different temperatures for a given schizophyllan−borax mixture ($C_p = 6$ g/L − $C_B = 15$ mM − NaCl 0.5 M). To keep the figure clear, only the curves corresponding to 1 Hz frequency are represented. Due to the gelation time observed, it was not possible to use temperatures above 25 °C, as the sample may then be damaged by dehydration. The gel time, t_{gel}, is shifted to larger values as the temperature increases; this result clearly shows that the physical functions responsible for the network formation are stabilized when the thermal agitation is lowered, indicating that the complex formation between the diol sites of the polysaccharide and the borate ions is an exothermic reaction.

Conclusion

In the presence of borate ions, schizophyllan shows a remarkable evolution of the viscoelastic properties, and in appropriate conditions, physical gel structures can result from the ionic complexation of the biopolymer. The rheological properties of the mixtures are largely affected by salt (content, nature) and temperature. The gel character is always observed after a time delay, thus indicating that the gelification corresponds to a connectivity transition. As the reaction rate is slow, it has been possible to use multifrequency oscillation experiments in order to study this system and to elucidate the behavior of schizophyllan−borax mixtures during the gelification reaction.

The scaling laws and self-similarity arguments well describe the sol-to-gel transition of this system; the corresponding parameters are in good accordance with the predicted values, for physical as well as chemical gels.

Further experiments are now performed to compare rheological and scanning electronic microscopy results, to give useful information about the morphology of the system for a better understanding of the gelification process of schizophyllan−borax mixtures.

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